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RESULTS OF AN ALGAL TOXICITY TEST APPLIED TO SEDIMENT ELUTRIATE--ETC(U)
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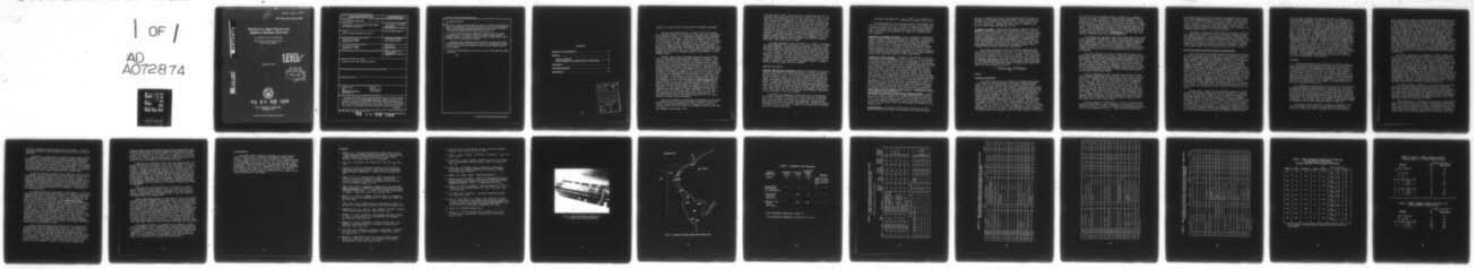

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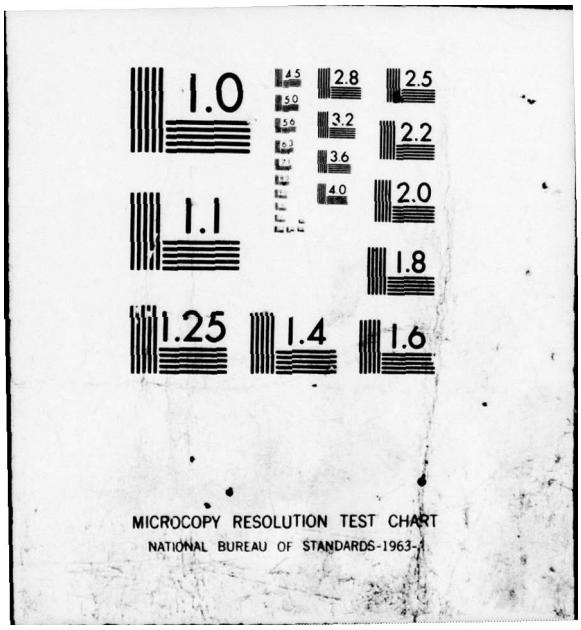
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NRL Memorandum Report 3952

Results of an Algal Toxicity Test Applied to Sediment Elutriates

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March 28, 1979

NAVAL RESEARCH LABORATORY
Washington, D.C.

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER NRL Memorandum Report 3952	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) RESULTS OF AN ALGAL TOXICITY TEST APPLIED TO SEDIMENT ELUTRIATES		5. TYPE OF REPORT & PERIOD COVERED Interim report on a continuing NRL problem. 6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Patrick J. Hannan and Constance E. Patouillet		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS Naval Research Laboratory Washington, D.C. 20375		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS NRL Problem 83G04-01
11. CONTROLLING OFFICE NAME AND ADDRESS Naval Research Laboratory Washington, D.C. 20375		12. REPORT DATE March 28, 1979 13. NUMBER OF PAGES 27
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)		15. SECURITY CLASS. (of this report) UNCLASSIFIED 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Sediment toxicity
Algae
Phaeodactylum tricornutum
Algal toxicity test
Elutriates
Thawed sediments
Frozen sediments

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Sediments from diverse marine and estuarine sites were subjected to a toxicity test based on the growth rate of the alga, Phaeodactylum tricornutum. Cultures were grown in solutions containing 1 part Guillard and Ryther medium and 11 parts sediment elutriate; growth was monitored by fluorescence measurements over a 2-day period. Comparisons with controls were made on the basis of a growth rate during the 20-44 hr interval, and on the final fluorescence of the cultures. In many instances there was a prolongation of the lag phase of growth but

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there were few cases in which the elutriates were highly toxic. A factor in the response of the algae was the concentration of the inoculum; with a heavy inoculum (over 30 ppm packed cell volume) a group of sediment elutriates promoted growth, but in the presence of very dilute inocula these same elutriates provided less growth than the controls.

It was found that some sediment elutriates did not reduce the toxicity of sublethal concentrations of Cu^{++}, but others did. Furthermore, prolonged storage of sediments at refrigerator temperatures (but unfrozen) decreased the capacity of certain sediments to reduce the toxicity of Cu^{++} when combined with elutriates.

Variations in toxicity of sediments, within close proximity to each other, were demonstrated with sediments from Puerto Mosquito Bay, Puerto Rico. Sediments from two stations in the Bay were not toxic, whereas sediment from a third site greatly inhibited the growth of the test organism.

There was good reproducibility in the results of this study, which extended over a period of three years.

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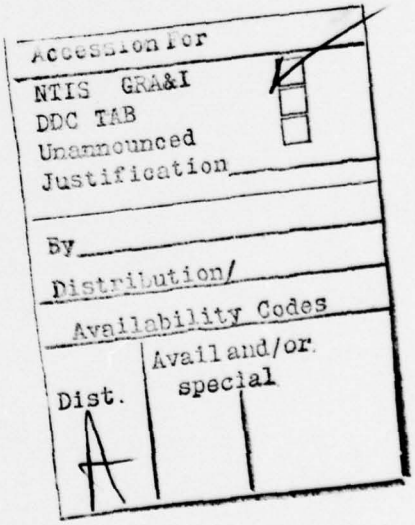

RESULTS OF AN ALGAL TOXICITY TEST APPLIED TO SEDIMENT ELUTRIATES

Although the potential threat to aquatic life from dredging and subsequent disposal of the spoils is being discussed increasingly in the literature there is no universally accepted thesis regarding the magnitude of the danger. Aside from the singular biases of the researchers involved, there is confusion resulting from the multiplicity of sediments; further, sediments can be of freshwater, estuarine, or marine origin, and their composition varies greatly in terms of grain size, organic content, heavy metals, and other properties. Also, the effects anticipated vary according to whether the potentially endangered species reside above, on, or within the sediment. For these reasons there often is an ambiguous answer to the question "Is this sediment toxic?"

Windom[1], among others, has stated that water quality changes cannot be predicted from bulk chemical analyses of sediments. Lee[2] has taken the position that little or no toxicity to water column organisms would be expected to arise from dredged material disposal. As an example of this, threespine sticklebacks and coho salmon fry showed no observable effect from 96-hr exposure to sediments from the Duwamish Waterway in Seattle, Washington, although high levels of contaminants were present[3]. Huggett et al[4] studied the Cu and Zn content of oysters in several estuaries and concluded that the higher concentrations were in organisms from fresher waters; the gradients found did not appear to be related to the distribution of these metals in the sediments. On the other hand Bryan and Hummerstone[5] found that the concentration of Cu in the polychaete, Nereis diversicolor, was roughly related to the total concentration in the sediment; the Zn content of the same organism remained remarkably constant despite wide variations in the environment, and appeared to be accurately regulated. A study made by Mathis and Cummings[6] indicated that Zn concentrations were highest in clams, intermediate in worms and lowest in fish fillets whereas with Cu, Ni, Pb, Cr, Li, Co, and Cd the concentrations were in the order worms >clams >fillets; the bottom-dwelling tubificids and clams closely reflected the concentrations of metals found in sediments.

One of the problems in correlating concentrations of pollutants in sediments and in the biota is the time lag between various processes. Young and McDermott-Ehrlich[7] found that the tissues of bottom-feeding fish in the region of Los Angeles County's Joint Water

Note: Manuscript submitted January 15, 1979.

Pollution Control Plant, off Palos Verdes, contained DDT and PCB's in concentrations higher than would have been expected following a 4-year reduction in the industrial input of these contaminants to the area. They concluded that contamination of sediments by these synthetic compounds caused them to persist in fish long after major reductions had been made in the dominant inputs; this conclusion was consistent with a laboratory study in which Dover sole maintained in clean, flowing, seawater and fed clean food nevertheless accumulated high levels of DDT and PCB when exposed to outfall zone sediments contaminated with these compounds. In a parallel study, Eganhouse and Young[8] indicated there was not a significant mobilization of Hg from sediments by Dover sole. Examples such as these provide a measure of the complexity of the dynamic equilibrium existing between elements in the water, sediment, and biota. Many others could be cited.

It seemed important to study the effect of a variety of sediments on a given organism as an indication of the range of toxicity one might find with sediments. Previous experience with algal cultures in this laboratory showed that reproducible data could be obtained with them[9, 10], both in terms of oxygen production and growth rates in the presence of a given toxicant; algae were therefore selected for this study. One advantage of their use is that toxic effects can be estimated in terms of percent inhibition of growth rates[11] rather than the all-or-nothing concept of lethality. For the growth rate determinations we chose to monitor fluorescence as a measure of chlorophyll content, a parameter shown to coincide roughly with cell counts[12].

MATERIALS AND METHODS

Organism: Phaeodactylum tricornutum Bohlin was maintained in stock cultures prepared with the f/2 medium described by Guillard and Ryther[13], and for 4 days before use it was subcultured in f/8 medium in 500 ml Erlenmeyer flasks at 23.0° ± 0.1°C while illuminated with fluorescent lamps. Artificial seawater was the base for this medium and was prepared from chemically pure salts and distilled water according to the formula of Lyman and Fleming[14] with the addition of 0.144 gm $NaHCO_3$/liter. Prior to their use the cells were centrifuged and washed three times with 3.5% NaCl to remove residual nutrients. They were then made into a slurry with 3.5% NaCl, and, after the chlorophyll content and packed cell volume (PCV) of the slurry had been determined, appropriate volumes were added to the test solutions.

Chlorophyll determinations of the slurry used for inoculating the cultures consisted in subjecting 2.0 ml of slurry to a heat shock (100°C for 1 1/2 min) followed by a cold shock (ice bath for several min), extraction with 3.0 ml CH_3OH and, ultimately, centrifugation. Absorbances at 650 and 665 nm were determined with a Beckman DU spectrophotometer, and the chlorophyll a content of the slurry was calculated from the following expression:

$$\text{micrograms chlorophyll/ml} = \frac{[(16.5 \times A^{665}) - (8.3 \times A^{650})] \times 2.}{3}$$

PCV determinations of the slurry were made by centrifuging a mixture, of 2.0 ml slurry and 8 ml 3.5% NaCl solution, for 15 minutes in a haematocrit made from a test tube fused to a thick-walled capillary tube. Calculations of the initial PCV of the test suspensions were based on the PCV of the slurry and the volume used to prepare each test suspension.

Preparation of sediment elutriates: After a sediment was measured in terms of a given volume, it was weighed and then added to a specified volume of seawater, shaken periodically for 30 minutes, centrifuged in plastic bottles to remove most of the particulates, and filtered through a 0.45μ filter (90 mm diam.) held in a Millipore Hydrosol filter (at 10 lbs. psi). The Hydrosol apparatus contained a plastic coated perforated disc as a support for the filter, a feature considered important in minimizing the adsorption of dissolved components in the filtrate because the fritted glass disc used in many filters is known to sorb appreciable amounts of minor constituents.

Composition of test cultures: A summary of the formulas for the cultures used in these experiments is shown in Table I. The concentration of nutrients was the same in each test, corresponding to f/24 of the Guillard and Ryther medium; also, each test included two concentrations of elutriate (referred to henceforth as Concentrated Elutriate and Half-Conc. Elutriate), two controls, and two samples containing Hg as a standard toxicant. The cell inocula were based initially on packed cell volume (PCV) and, later, on chlorophyll content. Hg concentrations used as a reference toxicant varied according to the inoculum used; for most of the experiments, which were started with 0.005 μgm chlorophyll/ml, the standard Hg concentration was 0.0033 ppm (mg/liter) but for the heavier inocula based on PCV the Hg concentration used was 0.01 ppm. At 0.005 μgm chlorophyll/ml, the inoculum would consist of approximately 10,000 cells/ml and would be approximately 3 ppm of PCV. The two concentrations of Hg were used simply for reference purposes without an intent to derive the same toxicity from each. The purpose in ultimately adopting the more dilute inoculum was to increase the sensitivity of the test method. A picture of an illuminated aquarium used in growing the cultures is shown in Fig. 1. Stirring was provided by the magnetic units underneath.

Preparation of suspensions containing the standard toxicant: To minimize errors caused by adsorption, certain precautions were necessary. The stock solution of $HgCl_2$ (1.353 gm/liter) was diluted tenfold just prior to use, and the required volume of this diluted stock was added to the test suspension immediately; as mentioned earlier, the concentrations used were either 0.010 or 0.0033 mg Hg/liter depending on the inoculum used.

pH measurements: An Orion meter (Model 801), connected to an Orion Manual Electrode Switch which can accommodate up to six electrodes,

was used to measure pH of many of the elutriates and of the test suspensions at the beginning of each experiment. Constant stirring was provided and each flask was stoppered to exclude free passage of air over the suspensions. Readings were taken until a constant pH was recorded, which often took more than an hour.

Assessment of growth: Fluorescence measurements formed the basis for estimates of the growth taking place in the test cultures. The procedure consisted in removing a 2 ml sample from each suspension, mixing it with 20 microliters of a 1 millimolar solution of 3-(3,4-dichlorophenyl)-1,1-dimethyl urea (DCMU), and measuring the fluorescence with a fluoro-microphotometer (American Instrument Co.) equipped with an R-136 photomultiplier tube and Corning glass filters No. 5543 and No. 2418. The procedure involving the use of DCMU provides a more linear relationship between fluorescence and chlorophyll content than is obtained with the normal fluorescence measurement[15].

For the test suspensions listed in Table II, comparisons with the controls were made solely on the basis of the relative fluorescences after a fixed period of 48 hours. For those included in Tables III-VIII the comparisons were based on two criteria: the fluorescence at the end of the test (44 hrs), and the growth rate calculated from fluorescence measurements made after 20 and 44 hrs. When an inoculum of 0.005 µgm chlorophyll/ml was used, by the time 20 hours had elapsed the cells were in the logarithmic phase of growth and the growth constant, "k", was calculated as follows:

$$k = \frac{\ln \text{Fluor}_{44\ \text{hrs}} - \ln \text{Fluor}_{20\ \text{hrs}}}{24}$$

RESULTS

Toxicity of elutriates

The initial experiments were intended to determine the response of the algae to elutriates prepared from several sediment/seawater ratios. The results obtained with six sediments from the Pacific (Table II) indicated that the lower sediment concentration gave essentially the same result as the higher; in these experiments growth in the elutriate-fortified suspensions was compared with the controls only on the basis of fluorescence at 48 hours. In each case the elutriates fostered growth, presumably because of the release of additional nutrients from the sediments. There was a problem in handling such dense sediment/seawater mixtures because of the long filtration times, even after an initial centrifugation of the slurries. The procedure was changed, therefore, to a lower sediment/seawater ratio and a decreased inoculum concentration (from a maximum of 37 ppm PCV to approximately 3 ppm, but based on 0.005 µgm chlorophyll/ml). There were several advantages to this new procedure: 1) less time was required to prepare the particulate-free elutriates, 2) with this lower inoculum concentration

the algae were in the exponential phase of growth for a convenient time period, making it possible to compute growth constants for each culture, and 3) the sensitivity of the method was increased. By comparing the growth of the elutriate cultures with the controls in terms of both the growth constants and the fluorescence at the end of a fixed time, a better insight into the effect of the elutriates could be obtained. The data summarized in Tables III-VIII were obtained with the one procedure involving the elutriation of 50 ml sediment with 950 ml seawater, and with an inoculum of Phaeodactylum cells such that their initial concentration was 0.005 μgm chlorophyll/ml.

An elutriate was considered toxic if the culture made from it had both a lower growth constant and a lower fluorescence value at the end of the experiment. In many cases, however, the elutriate suspensions had a higher growth rate but a lower fluorescence than the controls. This was interpreted to mean that the lag phase of growth was prolonged, resulting in a low fluorescence reading at both 20- and 44-hrs compared with the controls, but an elevated growth rate in the 20-44 hrs interval.

Table III summarizes the results obtained with sediments from marine sites, many being close to shore. These were mostly innocuous, the greatest effect on total growth being caused by segments of a core taken north of the mouth of the Orinoco River in Venezuela (Codes 19-21). However, another segment of that core (Code 22) was tested at a different time and had no effect; reference to the results obtained with the standard toxicant in each experiment indicated that the cells used in the earlier test were more sensitive, which probably accounted for the initial apparent toxicities. Factors causing such anomalies are discussed later in this report.

Table IV summarizes the data obtained with estuarine or freshwater sediments from diverse areas, the most notable result being the toxicity of a sediment from the bioluminescent Puerto Mosquito Bay in Puerto Rico. The initial test of this sediment (Code 12) suggested that it was toxic, and the repeat experiment (Code 14) verified the result. Sediments from other sections of the Bay were not toxic. Sediments from two sites in the New York Bight were tested (Codes 16 and 17) and the one composed of clean sand fostered growth while the black, fine-grained, muck in another caused a reduction in the final fluorescence. This result was confirmed in a later experiment (Code 18). In Tables III and IV, double horizontal lines are used to group together results obtained in a single experiment; in many instances there was only one sediment in a study encompassing assays of other pollutants such as oils or heavy metals.

Because salinity was considered to be a factor in the potential release of heavy metals in these experiments, it was desirable to determine the effect of a series of sediments having different salinities, such as those summarized in Table V. These came from Delaware Bay,

extending from the area near the ocean to the uppermost limits of the Bay. Three sediments appeared to be toxic according to the criteria of this test (C5B, F18B, and G2B); the toxic results with A2A and C8B could be ascribed to a reduced illumination of the cells in the elutriate suspension because of a heavy precipitate formed after the mixture of the elutriate with the culture medium. The locations of the sediments listed in Table V are shown in Figure 2.

The purpose in using two concentrations of elutriate in the sediment assays was to explore the effect of different sediment loadings in a disposal operation. In many instances both the concentrated and the half-concentrated elutriate provided approximately the same result, but in others there was a reversal of effects, e.g. Codes 12 and 13 in Table III, and 9 and 10 in Table IV; Table V lists many such changes between the concentrated and half-concentrated elutriates. Reference here is made to the final fluorescence values, not the "k" values.

Role of elutriates in reducing toxicity of heavy metals

One of the factors considered important in dredge spoils disposal has been the release of heavy metals but it has been shown by Davey et al[16] and others that metals are complexed by naturally occurring organic compounds, resulting in a decreased toxicity. To test this hypothesis with the algal assay described here, an elutriate of a Mare Island, CA, sediment was combined with Cu and Zn at concentrations normally inhibitory to algal growth. The first segment of Table VI summarizes the results obtained in this experiment, indicating that the toxicity of each metal was reduced appreciably in the presence of the diluted elutriate; even a 1/8 concentrated elutriate affected the toxicity of each. We should emphasize that in these experiments, the metal salt solution was not added to the sediments; it was added to the clear solution of filtered elutriate plus culture medium following the addition of algal cells. A partial repeat of this experiment with a sediment from the Pacific, summarized in Table VI, Section B, gave a different result indicating that the phenomenon was not to be expected in all cases. In the latter case, the concentrated elutriate reduced the toxicity only slightly and the more dilute solutions had even less effect.

For the sake of clarity the two experiments summarized in Table VII are shown together although they were not planned as a continuum. Because of the notoriety attached to the ecologically disastrous New York Bight dumping site, we had planned to use the black sediment (Table IV, Code 17) as the source of organic complexing material in an experiment designed to show that an elutriate could reduce the toxicity of a heavy metal. This was a prelude to a study in which sub-lethal concentrations of Cu were added to a culture, with subsequent analyses of the cells and the liquid phase, to determine the distribution of Cu as a function of time[17]. In the first experiment, summarized in Table VII, the elutriate effectively reduced the toxicity of Cu at either

0.4 or 0.5 ppm, even with the elutriate diluted by a factor of 12. Following that experiment, the sediment was left in the refrigerator unfrozen for approximately 7 weeks. The sequel (2nd experiment, Table VII) included only the 1/8 concentrated elutriate in the presence of 0.5 and 0.6 ppm Cu but the results were different than expected; there was no reduction of the Cu toxicity, suggesting that the characteristics of the sediment had changed during the time it was in the unfrozen (but cold) state. This result prompted the study with another sediment to determine whether thawing played a unique role in the performance of the sediment. A sediment taken from a site near New Guinea, which had been kept frozen since the time of collection, was compared with another sample of the same sediment which was in the thawed (refrigerated) state for 6 weeks. As shown in Table VIII, neither sample reduced the toxicity of either Cu or Zn in the manner previously expected. Perhaps the results would have been different if higher concentrations of elutriate had been used, but the results with the sediment from Mare Island had been so convincing that the experiment had been broadened in terms of toxicants rather than elutriate concentrations. These experiments suggest that there is a wide range of results to be anticipated when sediment elutriates are tested with sublethal concentrations of heavy metals.

DISCUSSION

The assays described in this report cover a period of approximately three years during which several large lots of synthetic seawater and multiple stock suspensions of algae were used. In many biological tests it is not unusual for the results to vary over such a long period, nevertheless the results of earlier tests compared well with those run at a later date. The relative importance of different batches of seawater, pH, and other factors are described elsewhere[10]; an important factor in such experiments is the care taken in minimizing the adsorption of trace constituents of elutriates to the walls of the culture vessels. For example, elutriates were used as soon after preparation as possible.

To illustrate the reproducibility of the method we call attention to several instances in which duplicate assays were run. Excellent agreement was obtained in Codes 3 and 4 of Table III and also in Table IV, Codes 12 and 14, and 17 and 18. In another instance of duplicate assays, with the Cape Hatteras sediments (Table III, Codes 12 and 13), the agreement was less satisfactory but partly explainable because the toxicities of the standard 0.0033 ppm Hg samples in these tests differed considerably. In the cases cited, the duplicate assays were performed at different times and with different sources of algal cells.

The purpose of this study was to broaden our knowledge of the effects of dredge spoils disposal on the organisms comprising the first level of the food chain. Sediments were collected from the

Pacific and Atlantic Oceans, the Caribbean and Mediterranean Seas, and from estuarine sites during a series of cruises on NRL research ships. To use a representative number of algal species in testing the effects of these sediments was prohibitive; therefore only one organism was used and the conclusions drawn from these studies must be limited in the light of that choice. Even with one organism the results can be diverse, depending upon the manner in which the tests were conducted. For example, under the conditions of the tests summarized in Table II (i.e. with a relatively dense inoculum) all the elutriates promoted growth, but when the elutriate of one of those same sediments was tested at an inoculum concentration approximately 1/5th that of the original (Table III, Code 32), there was a slight inhibition of growth. In a later experiment, with the greatly polluted sediment from Tokyo Bay, Japan, inocula ranging from 0.001 to 0.045 μgm chlorophyll/ml were used with the concentrated elutriate, and the progressive initial inhibition with decreased inoculum concentration became apparent. After 96 hours, however, all the cultures were approaching the same maximum fluorescence.

One unusual finding was the disparity of results obtained with elutriates of sediments taken from locations in close proximity in Puerto Mosquito Bay, Puerto Rico. Because of our interest in the bioluminescence of this bay, samples were taken at three sites ---- one was toxic but the other two were not. A repeat of the experiment with the toxic sediment gave the same result (Table IV, Codes 12 and 14). Because of its shallow depth, less than 2 meters in most places, and the influence of freshwater and seawater incursions there is a great dynamism in the composition of the bay. Analyses of several water samples were made, for example, and while the surface water at Station "A" contained 0.032 μgm at P/liter the water just 1 meter below had an undetectable amount. The distance from Station "A" to the others was approximately 700 meters. A similar study was made of three locations in Oyster Bay, Falmouth, Jamaica, which was another site of bioluminescence, and only one of the three showed any toxicity.

In several instances, segments of cores from a single site were tested and in general the results were the same for each portion. Table III contains an apparent contradiction in that three portions of a core taken from the Cariaco Trench promoted growth but others did not; these results can be explained, however, in that the standard toxicant (0.0033 ppm Hg) caused less inhibition in the first instance than the others, indicating that the particular batch of cells used was less susceptible for reasons not known.

Growth enhancement by elutriates occurred often in these experiments, presumably because of the release of nutrients from the sediments. In at least one instance, with a sediment taken from the area near Puerto LaCruz, Venezuela, there was a considerable increase in growth which was anticipated; the chlorophyll content of the surface waters taken at the time the sediment was collected was 8 mg/m^3 and

the water contained an enormous population of anchovies. It was not surprising, therefore, that sediments from that site would be rich in nutrients.

Complexities in predicting the effects of dredge spoils disposal are apparent in a comparison of the contrasting effects obtained in the algal assays with concentrated and half-concentrated elutriates. Reverse effects of the two concentrations could be seen in seven of the eleven sediments from Delaware Bay and, among others, the sediments from Cape Hatteras, Oyster Bay, and the New York Bight. It is possible that each reverse effect represents a response to toxic or growth promoting entities which change considerably with concentration.

We had not anticipated changes in the complexing characteristics of sediments after being thawed from the frozen state. In view of the premise that sediments should be stored at 4°C, and not frozen, it would be interesting to compare the effects of a given sediment before freezing and after freezing; from the results cited here there are indications that sediments stored unfrozen in the refrigerator change considerably but the freezing beforehand may have been responsible for the rupture of some microorganisms in the sediments. This point has not been investigated.

In connection with the complexation of heavy metals, it should be mentioned that natural waters and elutriates of sediments often contain organics which affect the activity of metal ions. Recently Sunda and Lewis[18] correlated the complexation of Cu by organic ligands in river water with the reduction of toxicity toward Monochrysis lutheri. These results were consistent with several previous studies which indicated that Cu^{++} was the chemical species responsible for the toxicity of copper, and they also supported the general thesis that suspended sediments, as a source of organic compounds, would reduce the toxicity of metal salts. In the studies reported here, however, there were several instances in which sediments did not reduce the toxicity of heavy metals (Table VI, part B; Table VII, 2nd section; and Table VIII) indicating that generalizations may be hazardous in this matter. It is reasonable to suppose that a sediment having a low cation exchange capacity, such as sandy material, would not be effective in reducing the toxicity of heavy metals but the sediments used in these particular studies were not predominantly sandy.

A major concern in dredge spoils disposal is the translocation of heavy metals from the sediments into the biota. This study has not addressed that question directly although many samples of algal cells grown in the presence of elutriates have been collected and await analyses. It had been shown here earlier[19] that Hg^{203} which was sorbed to a variety of sediments was not leached in detectable concentrations by fresh seawater. This was not surprising since Hg forms such strong bonds with sulfur, for example, which occurs in both inorganic and organic forms in sediments. Related to the problem of heavy metal

transfer through the ecosystem was a study of marsh plants which indicated that heavy metals are seldom transferred in significant amounts to the leaves and stems of these plants[20]. In another study of marsh plants[21], the uptake of Zn, Cu, and Cd could be predicted on the basis of extraction of the sediment by diethylene triamine penta-acetic acid (DPTA), but the correlation did not hold with Ni or Hg.

Having devoted considerable time to the study reported here, and having found that most sediments are not toxic according to the conditions of the algal test used, an overview of the project is certainly in order. Probably the principal result is the conviction that the disposal of dredged sediments will generally not be deleterious, from a chemical standpoint, to the primary producers in the sea; while not a part of this study, it is possible that the turbidity resulting from spoils disposal will play a more obvious role on photosynthesis than the release of constituents from the sediments. Extensive monitoring of heavy metals in the water and in benthic macroinvertebrates along the New England Coast has indicated no substantial change as the result of dredging[22].

Aside from the rough determination of the percent of organic matter contained in these sediments, there has been no study of this factor. It would be interesting to investigate the cation exchange capacity, particularly of the sediments known to have been altered by a prolonged time in the thawed state.

Results with algal assays do not engender the type of zeal asscciated with ecologists' demands that certain actions be taken to preserve species higher up in Nature's pecking order. They can be useful, however, in detecting certain types of pollution; also in the studies here the algae provided a handy tool to detect the variety of responses which might result from various sediment/seawater/inoculum combinations. For decisions to be made regarding a particular dredge spoils disposal site it is conceivable that algae could serve a very useful purpose. Research described by Kayser illustrates the point[23]. He studied the long term effect of "red muds" on the growth of algae and in most of the batch cultures used the algae grew at reduced rates initially but eventually grew as well as the controls. In continuous cultures, however, with daily additions of the "red muds" the growth rate was reduced.

Bulk chemical analyses of sediments are not likely to be particularly informative on an ecological basis, and even chemical analyses of a water column, without a knowledge of the chemical speciation, can be misleading. It is in these contexts that a biological assay becomes imperative. An advantage of the biological assay based on algae is that it can be done quickly and reproducibly, and the number of variables to be considered can be manipulated rather easily.

ACKNOWLEDGMENTS

The authors wish to thank R.A. Gallatin, G.W. Mullis, and R. Phelan for their help in collecting the sediments during recent cruises. Thanks are due also to R.E. Nekritz for the characterization of certain samples, and to L.H. DiSalvo, J.B. Pearce, and R.C. Malloy for the sediments from Oakland Bay, New York Bight, and Mare Island respectively. Thanks are due also to Brenda Little for the sediments from Mayport, Fla., and to J.F. Wehmiller for those from Delaware Bay. We are particularly indebted to D.W. Strasburg and C.H. Cheek for their planning of the NRL research cruises and to R.A. Neihof for his critical evaluation of this report.

REFERENCES

1. Windom, H.L., "Processes Responsible for Water Quality Changes During Pipeline Dredging in Marine Environments", prepared under contract (DACW-21-71-C-0020) between the U.S. Army Corps of Engineers and Skidaway Institute of Oceanography.

2. Lee, G.F., Discussion, Jour. Water Poll. Contr. Fed. 49, 1920, 1977.

3. LeGore, R.S. and D.M. DesVoigne, "Absence of Acute Effects on Threespine Sticklebacks and Coho Salmon Exposed to Resuspended Harbor Sediment Contaminants", J.Fish. Res. Bd. Can. 30, 1240, 1973.

4. Huggett, R.J., R.A. Cross, and M.E. Bender, "Distribution of Cu and Zn in Oysters and Sediments from Three Coastal Plain Estuaries" presented at Symposium on Mineral Cycling in Southeastern Ecosystems, Athens, Ga. 1974.

5. Bryan, G.W., and L.G. Hummerstone, "Adaptation of the Polychaete Neris diversicolor to Estuarine Sediments Containing High Concentrations of Heavy Metals. I. General Observations and Adaptation to Copper", J. Mar. Biol. Assn. U.K., 51, 845, 1971.

6. Mathis, B.J., and T.F. Cummings, "Selected Metals in Sediments, Water and Biota in the Illinois River", J. Water Poll. Contr. Fed. 45, 1573, 1973.

7. Young, D.R., and D. McDermott-Ehrlich, "Sediments as Sources of DDT and PCB", Coastal Water Research Project, Annual Report, 1976.

8. Eganhouse, R.P., Jr., and D.R. Young, "Mercury in Benthic Animals", Coastal Water Research Project, Annual Report, 1976.

9. Hannan, P.J., and C. Patouillet, "Gas Exchange with Mass Cultures of Algae. II. Reliability of a Photosynthetic Gas Exchanger", Appl. Microbiol. 11, 450, 1963.

10. Hannan, P.J., and C. Patouillet, "An Algal Toxicity Test, with Emphasis on Adsorption Effects", accepted for publication by Jour. Wat. Poll. Cont. Fed.

11. Hood, D.W., T.W. Duke and B. Stevenson, "Measurement of Toxicity of Organic Wastes to Marine Organisms", J. Water Poll. Contr. Fed. 32, 982, 1960.

12. Kayser, H., "Waste Water Assay with Continuous Algal Cultures: The Effect of Mercuric Acetate on the Growth of Some Marine Dinoflagellates", Mar. Biol. 36, 61, 1976.

13. Guillard, R.R., and J.H. Ryther, "Studies on Marine Planktonic Diatoms", Can. J. Microbiol. 8, 229, 1962.

14. Lyman, J., and R. Fleming, "Composition of Seawater", J. Mar. Res. 3, 134, 1940.

15. Slovacek, R., and P.J. Hannan, "In vivo Fluorescence Determinations of Phytoplankton Chlorophyll a", Limnol. & Oceanog. 22, 919, 1977.

16. Davey, E.W., M.J. Morgan, and S.J. Erickson, "A Biological Measurement of the Copper Complexation of Seawater", Limnol. and Oceanogr. 18, 993, 1973.

17. Hannan, P.J., and A. Zirono. Unpublished results.

18. Sunda, W.G. and J.M. Lewis, "Effects of Complexation by Natural Organic Ligands on the Toxicity of Copper to a Unicellular Alga Monochrysis lutheri", Limnol. & Oceanogr. 23, 870, 1978.

19. Hannan, P.J. and N.P. Thompson, "Uptake and Release of ^{203}Hg by Selected Soil and Sediment Samples", J. Wat. Poll. Contr. Fed. p. 842, May 1977.

20. U.S. Army Corps of Engineers. Information Exchange Bulletin. Vol. D-77-8, Aug. 1977.

21. Lee, C.R., R.M. Smart, T.C. Sturgis, R.N. Gordon, Sr., and M.C. Landin, "Prediction of Heavy Metal Uptake by Marsh Plants Based on Chemical Extraction of Heavy Metals by Dredged Material", Tech. Report D-78-6, U.S. Army Corps of Engineers, Feb. 1978.

22. Brown, C.L., Personal communication.

23. Kayser, H., "Über den Einfluß von Rotschlamm auf die Kultur einiger mariner Planktonalgen. Helgolan wiss. Meeresunters, 25, 357, 1973.

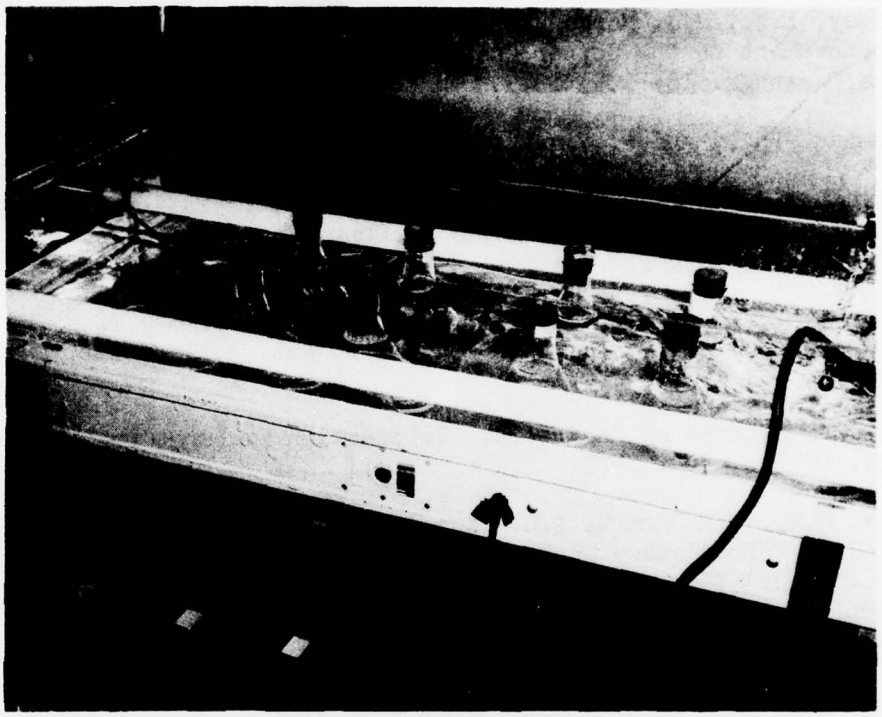

Fig. 1 — Illuminated aquarium containing up to 10 cultures; two such aquaria were used.

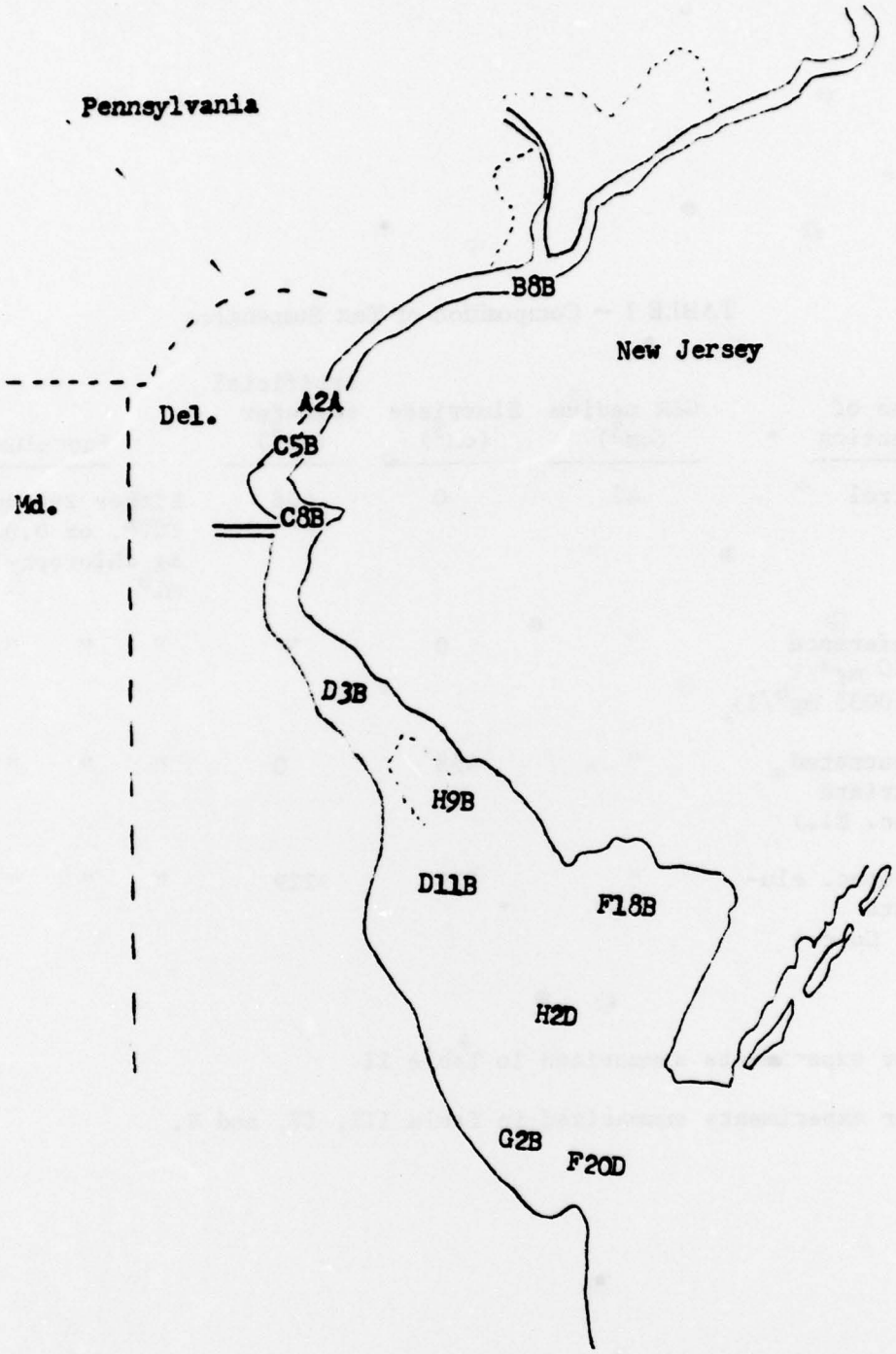

Fig. 2 — Location of sediment samples from Delaware Bay

TABLE I — Composition of Test Suspensions

Type of suspension	G&R medium (cm^3)	Elutriate (cm^3)	Artificial seawater (cm^3)	Inoculum
Control	42	0	458	Either 29 ppm PCV[a], or 0.005 mg chlorophyll/ml[b]
Hg reference (0.010 mg[a]/l or 0.0033 mg[b]/l)	"	0	"	" " "
Concentrated elutriate (Conc. El.)	"	458	0	" " "
Half-Conc. elutriate (1/2 Conc.)	"	229	229	" " "

[a]For experiments summarized in Table II

[b]For experiments summarized in Table III, IV, and V.

TABLE II — Effect of Elutriates of Sediments from Pacific Ocean on Growth of Algae (48 Hrs) Following Inoculum of 29 ppm Cells

A) Sediment Concentration = 70 ml/liter Seawater

Code	Site	Depth (M)	Description	Dry Wt. Mixed with Seawater (gm/l)	% Organic in Sediment	Initial pH of Elutriate Suspension	Elutriate Effect on Algal Growth (%)	0.010 ppm Hg Effect on Algal Growth
A	3°33'S 144°16'E	730	Dark gray clay, slightly calcareous; slightly gritty	38	9.4	8.32	+ 48	
B	4°05'S 145°39'E	1340	Dark gray clay, slightly calcareous; slight HCl reaction	44	8.9	8.39	+ 31	
C	3°51'S 144°33'E	30	Dark gray clay; no HCl reaction	46	8.6	8.09	+ 21	- 22 - 26
D	3°41'S 144°20'E	150	Dark gray clay, slightly gritty; no HCl reaction	60	6.6	8.38	+ 31	pH = 8.21
E	3°26'S 144°27'E	1260	Dark gray clay, slightly calcareous	32	10.5	8.28	+ 42	
F	Yokosuka Pier	6	Black clay; oily odor; no HCl reaction	18	18.4	8.66	+ 25	
B) Sediment Concentration = 150 ml/liter Seawater								
A	See above for location, depth, etc.			86	See Above	8.40	+ 35	
B	" " " " " "			96	"	8.48	+ 37	
C	" " " " " "			105	"	8.15	+ 56	- 19 - 21
D	" " " " " "			148	"	8.35	+ 35	pH = 8.32
E	" " " " " "			71	"	8.34	+ 47	
F	" " " " " "			36	"	8.82	+ 34	

TABLE III — Effect of Elutriates of Sediments from Various Marine Sites on Algal Growth (44 Hrs) Following Inoculation with Cells at 0.005 Microgram Chlorophyll/ml

Code	Coordinates	Location	Depth (M)	Sediment Description	% Organic	Elutriate pH	Culture	% Change According to: k	% Change According to: Fluor.
1	10°47'N 60°41'W	West of Trinidad	62	Mostly sand; some red coloration	7.1	8.11 8.10	Conc. 1/2 conc	+3 +7	-6 +17
2	10°16'N 64°46'W	Near Puerto La Cruz, Venezuela	49	Greenish, smooth paste; overlying water contained large concentration of Phaeocystis	9.9	8.28 8.19	Conc. 1/2 conc	+7 +13	+5 +25
3	17°54'N 67°04'W	South of Ponce, Puerto Rico	15	White sand containing some red coral	6.6	8.12 8.11	Conc. 1/2 conc	-1 +6	-8 +11
4	17°54'N 67°04'W	South of Ponce, Puerto Rico	15	White sand containing some red coral	6.6	N.D. N.D.	Conc. 1/2 conc	-4 +5	0 +9
5	10°41'N 65°36'W	Cariaco Trench	1340	Grayish green mud; Strong H_2S smell 0-10 cm	N.D.	8.00 8.00	Conc. 1/2 conc	+9 +12	-10 +1
6	10°41'N 65°36'W	" "	1340	" " " " " " 40-50 cm	14.6	8.04 8.04	Conc. 1/2 conc	+30 +19	-9 -7
7	10°41'N 65°36'W	" "	1340	" " " " " " 110-120 cm	N.D.	8.11 8.07	Conc. 1/2 conc	+35 +12	-14 +4
8	10°41'N 65°36'W	" "	1340	" " " " " " 20-30 cm	N.D.	8.07 8.05	Conc. 1/2 conc	+18 +11	+12 +7
9	10°41'N 65°36'W	" "	1340	" " " " " " 30-40 cm	14.2	8.06 8.05	Conc. 1/2 conc	+20 +8	+9 +5
10	10°41'N 65°36'W	" "	1340	" " " " " " 90-100 cm	11.8	8.08 8.05	Conc. 1/2 conc	+33 +14	+25 +28
11	18°30'N 77°38'W	Falmouth, Jamaica	16	Essentially broken shells; light color	3.4	8.05 8.01	Conc. 1/2 conc	+6 +6	+7 +13
12	35°28'N 75°10'W	Cape Hatteras	31	Very fine black sand	2.3	8.17 8.14	Conc. 1/2 conc	+3 +11	-4 +17
13	35°28'N 75°10'W	" "	31	" " " "	N.D.	8.17 N.D.	Conc. 1/2 conc	+2 +6	-27 0
14	41°48'N 8°54'E	Southwest of Ajaccio, Corsica	15	Dark, smooth, mud containing some plant material Sal = 40.9	10.1	8.07 N.D.	Conc. 1/2 conc	0 +4	-20 -2
15	41°48'N 8°52'E	" " " "	305	Dark, smooth, mud	13.7	N.D. N.D.	Conc. 1/2 conc	+13 +15	-16 -2

16	37°08'N 20°25'E	West of Peloponnesus	500	Brown mud	12.4	8.10 N.D.	Cone. 1/2 cone	+6 +4	-20 -6
17	39°53'N 14°36'E	South of Gulf of Salerno	1275	Dark brown clay; slightly coarse	14.0	8.13 N.D.	Cone. 1/2 cone	+7 +10	-6 -3
18	40°22'N 14°16'E	Gulf of Salerno	850	Dark brown clay; slightly coarse	12.5	8.11 N.D.	Cone. 1/2 cone	+14 +22	-16 +2
19	9°00'N 60°12'W	North of Mouth of Orinoco River	35	Light gray clay 0-10 cm	8.4	8.37 8.24	Cone. 1/2 cone	+4 +3	-41 -30
20	9°00'N 60°12'W	" " " " " "	35	" " " 60-70 cm	N.D.	8.37 8.25	Cone. 1/2 cone	+16 0	-39 -30
21	9°00'N 60°12'W	" " " " " "	35	" " " 120-130 cm	8.0	8.27 8.16	Cone. 1/2 cone	+13 +7	-41 -24
22	9°00'N 60°12'W	" " " " " "	35	" " " 20-30 cm	9.1	8.52 N.D.	Cone. 1/2 cone	+23 +19	-3 +3
23	10°15'N 60°46'W	West of Trinidad	44	Coarse sand and grayish clay	10.7	N.D. N.D.	Cone. 1/2 cone	+5 +3	+7 +7
24	23°08'N 74°50'W	Crooked Island Passage, Bahamas	50	Coarse, yellowish, coral sand 10-20 cm	2.8	8.18 N.D.	Cone. 1/2 cone	+3 +7	+19 +15
25	23°08'N 74°50'W	" " " "	50	" " " " 45-55 cm	2.2	8.18 N.D.	Cone. 1/2 cone	+3 +5	+12 +8
26	23°08'N 74°50'W	" " " "	50	" " " " 65-75 cm	4.2	8.20 N.D.	Cone. 1/2 cone	+4 +3	+19 +16
27	10°20'N 62°16'W	Off Puerto La Cruz, Venezuela	28	Greenish, smooth paste; high chlorophyll concentration in overlying water 10-20 cm	8.0	8.16 8.12	Cone. 1/2 cone	+10 -6	0 -2
28	10°20'N 62°16'W	" " " " "	28	" " " " " 20-30 cm " " " " "	8.8	8.40 N.D.	Cone. 1/2 cone	+26 +10	+1 +3
29	11°01'N 65°49'W	From the sill of the Cariaco Trench	80	Sandy with yellowish caste; abundant foraminifera	17.6	8.20 N.D.	Cone. 1/2 cone	+8 -4	-2 -5
30	37°34'N 23°37'E	Northwest of Poros, Greece	154	Mostly sand but with some clay and small shells	7.0	8.20 N.D.	Cone. 1/2 cone	+3 +7	-4 +6
31	36°35'N 21°11'E	Southwest of Milos, Greece	5100	Brown mud with some sand and small shells	16.5	8.12 N.D.	Cone. 1/2 cone	0 +6	-13 -4
32	3°41'S 144°20'E	Northwest of Madang, New Guinea	150	Dark gray clay; slightly calcareous	6.6	8.51 N.D.	Cone. 1/2 cone	-1 N.D.	-17 N.D.

N.D. indicates "Not Determined".

TABLE IV — Effect of Elutriates of Sediments from Various Coastal and Estuarine Sites on Algal Growth Following Inoculation with Cells at 0.005 Microgram Chlorophyll/ml

Code	Location	Depth (M)	Sediment Description	Interstitial Salinity	% Organic	Elutriate pH	Culture	% Change According to: k	% Change According to: Fluor
1	Mare Island, CA #23S 12/28/76	10	Smooth gray mud, no shells or odor	26.4	9.5	N.D.* N.D.	Conc. 1/2 conc	+4 0	+7 +10
2	" " " #22S 12/28/76	10	" " " " " " "	21.5	10.0	N.D. N.D.	Conc. 1/2 conc	+6 +4	+15 +8
3	" " " #23S (A) 2/11/77	10	" " " " " " "		9.3	8.00 8.09	Conc. 1/2 conc	+4 0	-10 +6
4	" " " #23S (B) 2/11/77	10	" " " " " " "			7.98 8.05	Conc. 1/2 conc	+9 0	-6 +3
5	" " " #23S (C) 2/11/77	10	" " " " " " "	19.5	8.6	8.08 8.13	Conc. 1/2 conc	+11 +1	0 0
6	Mayport, Fla.	10	Black, fine grained muck	34.5	14.3	8.14 N.D.	Conc. 1/2 conc	+24 +16	+2 +6
7	Oyster Bay, Puerto Rico Sta. 1.	<2	Mostly blackish sand, with some shells and pebbles	33.2 30.0	11.4 20.2	8.05 8.02	Conc. 1/2 conc	+7 +10	+2 +12
8	" " " " Sta. 2	<2	Similar to above, but with more shells	31.1	13.5	8.01 7.99	Conc. 1/2 conc	+6 +7	+1 +16
9	" " " " Sta. 3 (Middle section)	<2	Fine-grained, black slurry; slight fishy smell	35.0	22.9	8.16 N.D.	Conc. 1/2 conc	+13 +4	-19 0
10	" " " " Sta. 3 (Top section)	<2	Black sand and clay, with small shells	33.8	21.9	N.D. N.D.	Conc. 1/2 conc	+12 +8	-3 +13
11	" " " " Sta. 3 (Bottom section)	<2	Mostly gray clay, with few small shells	34.2	20.5	N.D. N.D.	Conc. 1/2 conc	+17 +1	+15 +3
12	Puerto Mosquito Bay, P.R. Sta. A.	<2	Black sand with shells and organic debris	5.9	28.0	N.D. N.D.	Conc. 1/2 conc	-9 -19	-41 -40
13	" " " " Sta. B.	<2	Black sand with many shells	35.0	8.0	N.D. N.D.	Conc. 1/2 conc	+7 +7	+13 +12
14	" " " " Sta. A.	<2	Black sand with shells and organic debris	5.9	28.0	8.18 N.D.	Conc. 1/2 conc	-14 -37	-48 -53
15	" " " " Sta. C.	<2	Gray mud with few small shells; no odor	40.3	16.9	8.22 N.D.	Conc. 1/2 conc	+3 +3	-4 +6
16	New York Bight "Control site"	40	Clean sand	22.5	1.4	N.D. N.D.	Conc. 1/2 conc	+20 +18	+15 +17
17	" " " "Disposal site"	40	Black, fine-grained muck	27.5	7.8	N.D. N.D.	Conc. 1/2 conc	+20 +13	-19 +7
18	" " " "Disposal site	40	Black, fine-grained muck	"	"	N.D. N.D.	Conc. 1/2 conc	+7 +14	-13 +20
19	Yokosuka, Japan (Harbor)	15	Black, fine-grained muck with no shells; oily; foul smell	42.3	15.5	8.51 N.D.	Conc. 1/2 conc	-1 -3	-11 -6

*N.D. indicates "Not Determined".

TABLE V — Effect of Elutriates of Sediments from Delaware Bay on Algal Growth (44 Hrs) Following Inoculation with Cells at 0.005 Microgram Chlorophyll/ml

Name	% Sal.	% Dry Wt.	% Org.	pH Conc. El	Effect according to:	k	Fluor
A2A	1.42	38.8	8.7	6.91*	Conc.	-53	-86
					½ conc.	-12	-37
C5B	1.70	47.5	5.8	7.83	Conc.	-11	-38
					½ conc.	+12	-6
B8B	1.78	75.7	0.5	8.05	Conc.	+9	-24
					½ conc.	+9	+7
C8B	6.65	53.3	6.2	7.29*	Conc.	-28	-71
					½ conc.	-3	-22
D3B	8.58	52.1	6.7	7.91	Conc.	+19	-7
					½ conc.	+27	-3
D11B	17.16	77.5	1.3	8.18	Conc.	+23	-2
					½ conc.	+13	+10
H9B	22.09	69.6	2.6	8.16	Conc.	?	-19
					½ conc.	+4	+3
F20D	24.24	65.3	3.1	8.21	Conc.	+23	-45
					½ conc.	+16	0
H2D	24.8	83.9	.7	8.10	Conc.	0	-16
					½ conc.	+3	+5
F18B	26.54	76.5	1.0	8.17	Conc.	-13	-84
					½ conc.	-7	-26
G2B	33.96	74.1	1.5	8.18	Conc.	?	-92
					½ conc.	-1	-26

*Yellow flocculant, precipitate gradually formed when elutriate was mixed with culture medium.

TABLE VI. Section A — Effect of Elutriate of Sediment from Mare Island, CA, on Toxicity of Copper and Zinc

Culture	% Change according to: "k"	Final fluor.
Conc. elutriate	+11	0
1/2 " "	+1	0
Cu, 0.4 ppm	-25	-49
Zn, 7.0 "	-57	-72
Cu, 0.4 ppm + Conc. elut.	+3	-7
" " " + 1/2 " "	-1	-5
" " " + 1/4 " "	0	-13
" " " + 1/8 " "	-6	-15
Zn, 7.0 ppm + Conc. elut.	+5	-1
" " " + 1/2 " "	-4	-5
" " " + 1/4 " "	-5	-15
" " " + 1/8 " "	-5	-16

Section B — Effect of Elutriate of Sediment from Pacific Ocean (3°41'S, 144°20'E) on Toxicity of Zinc

Culture	% Change according to: "k"	Final fluor.
Conc. elutriate	-1	-17
Zn, 7.0 ppm	-77	-86
Zn, 7.0 ppm + Conc. elut.	-46	-74
" " " + 1/2 " "	-57	-75
" " " + 1/4 " "	-59	-81
" " " + 1/10" "	-70	-85

TABLE VII — Effect of Elutriates of New York Bight Sediment on Toxicity of Copper

1st Experiment, with sediment in frozen state until the time of the test:

Culture	% Change according to: "k"	% Change according to: Final fluor.
Conc. elutriate	+7	-13
1/2 " "	+14	+20
1/4 " "	+14	+20
1/8 " "	+8	+13
Copper, 0.40 ppm	-21, -20	-29, -29
" 0.50 "	-27, -18	-44, -36
1/2 Conc. elutriate plus 0.40 ppm Cu	-8	-11
1/4 " " " " " "	-9	-18
1/8 " " " " " "	-12	-22
1/12 " " " " " "	-8	-16
1/2 Conc. elutriate plus 0.50 ppm Cu	-6	-18
1/4 " " " " " "	-8	-18
1/8 " " " " " "	-15	-27
1/12 " " " " " "	-1	-7

2nd Experiment, with sediment in thawed state for 7 weeks prior to the test:

Culture	% Change according to: "k"	% Change according to: Final fluor.
1/8 Conc. elutriate	+5	+13
Copper, 0.6 ppm	-47	-57
1/8 Conc. elutriate plus 0.6 ppm Cu	-42, -44	-68, -66
" " " " 0.5 " "	-38, -47	-57, -57

(The culture containing 0.5 ppm Cu alone was accidentally destroyed)